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**COMPOSITION OF COAL DUSTS AND THEIR
CYTOTOXICITY ON ALVEOLAR MACROPHAGES**

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13. ABSTRACT (Maximum 200 words) Coal mine dust is produced from complex materials consisting of organic sedimentary strata, inorganic minerals, and trace elements. The dust varies in its chemical compositions and is capable of causing lung injury and damage when inhaled. The purpose of this study was to perform scanning electron microscopy combined with energy-dispersive spectrometry, wavelength-dispersive spectrometry, and X-ray diffraction analyses of three coal dusts, and examine their effects on rat lung alveolar macrophages (AMs) in cell culture. The coal dusts were obtained from coal surfaces of anthracite, meager, and fat coal mines. The AMs were harvested in bronchoalveolar lavage from adult male Wistar rats and were cultured in Eagle's medium at 37°C. Prostaglandin E ₂ (PGE ₂) and lactate dehydrogenase (LD) released by cultured AMs were measured by radioimmunoassay and enzymatic methods, respectively, 24 hours after addition of coal dust. Elemental analysis revealed that all dusts consisted of high carbon content (93-96 wt%); small amounts of Si, Al, Ca, S, Mg, Ti, Na, Fe, K; and trace elements. Anthracite contained the highest sulfur concentration and fat coal the highest calcium concentration. Mineralogic analysis by X-ray diffraction revealed the existence of high amorphous carbon background. Kaolinite with chemical form Al ₂ Si ₂ O ₅ (OH) ₄ was the major mineral component in all three coal dusts. In addition, meager coal contained muscovite, anthracite contained pyrite and muscovite, and fat coal contained calcite. Cytotoxicity was evident in AM culture of all three coal dusts, which caused the release of LD and PGE ₂ . The release was dose-dependent. In summary, our study shows that all three coal dusts exhibit cytotoxicity to AMs and suggests that the pathogenesis of coal associated with pulmonary disease may be linked to the elemental compositions and mineralogic components.					
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INTRODUCTION

Coal mine dust is produced from complex materials consisting of organic sedimentary strata, minerals, and trace elements. The dust varies in its chemistry, depending on its source; i.e., location of the mine, the type of coal-anthracite vs. bituminous. The dust is generated from pulverized rocks associated with the coal. It can cause a tissue reaction when inhaled, ranging from a mild inflammatory response to coal workers' pneumoconiosis (CWP).^[1] As with other forms of pneumoconiosis, the degree of host response depends partly on the amount of dust inhaled, the duration of exposure, the rank of coal, and the host cellular response.^[2] The purpose of this study is to examine three coal mine dusts from different mines in China to determine their elemental and mineral compositions and the effects on alveolar macrophages (AMs). The AMs are the principal cells involved in the defense of the lung to inhaled dusts and play a central role in the pathogenesis of dust-related tissue injury. In addition to their phagocytic function, they are capable of releasing various cytokines, enzymes, and growth factors many of which are mediators relevant to the pathogenesis of chronic inflammation and fibrosis.^[3]

EXPERIMENTAL PROCEDURE

Animals

The AMs were obtained from male Wistar rats throughout the study. Rats weighing 250-300 grams were housed in wire cages and received food and water *ad libitum*. The rats remained healthy without apparent signs of infection.

Bronchoalveolar Lavage

After anesthesia, the rat's trachea was cannulated, and the heart and lungs removed en bloc. The AMs were harvested by bronchoalveolar lavage by instilling five 10-ml boluses of sterile physiological saline. The saline was withdrawn into a syringe following gentle massaging of the lung and the retrieved boluses pooled.

Alveolar Macrophage Cultures

After harvest, the AMs were adjusted to 1×10^6 /ml in Eagle's medium with antibiotics plus 10% heat-inactivated fetal calf serum; 2×10^6 cells were placed in sterile glass tissue culture flasks. After one hour of incubation at 37°C in a 5% CO₂/95% humidified air atmosphere, the nonadherent cells were removed by two washings with Hank's medium. Each culture then received 2.0 ml of Eagle's medium without fetal calf serum, to which was added coal dust so that the final dust concentration was 200 µg/ml, 400 µg/ml, or 800 µg/ml. Four or five macrophage/coal dust cultures were incubated at 37°C in a 5% CO₂/95% humidified air atmosphere. Viability was determined by trypan blue dye exclusion.

Coal Dust Preparation

Coal samples were obtained from the coal face of anthracite, meager, and fat coal mines. After passing through the crusher at the mine site, the coal samples were ground to a fine powder using an agate mortar and pestle. The powders were passed through a 200-mesh sieve to allow passage of particles less than 74 μm . Meager and fat are Chinese terms for a bituminous coal used for industrial purposes including the generation of electrical power. Anthracite coal is typically used for home heating and cooking.

Elemental Composition of Coal Dust

The elemental composition of the coal samples was determined by scanning electron microscopy in combination with energy-dispersive X-ray spectrometry and wavelength-dispersive X-ray spectrometry. For these studies, the following equipment was used: JEOL 6100 scanning electron microscope (JEOL USA, Inc., Peabody, MA) equipped with a Fison Kevex Delta-Pro energy-dispersive X-ray spectrometer (Fison Corp., San Carlos, CA) and a Rigaku X-ray fluorescence spectrometer (Danvers, MA). X-ray spectra were collected from a minimum of 50 individual coal particles from each coal type.

X-Ray Diffraction Analysis

The three coal samples were analyzed using a Scintag X-ray diffractometer (Scintag, Inc., Sunnyvale, CA). The diffractometer was equipped with a θ -2 θ goniometer with 0.0003 degree resolution, and a diffractometer radius set at 286 mm. A Kevex-Peltier-cooled Si(Li) solid-state detector with a microprocessor-controlled four-axis microstep diffractometer motion was used with a multichannel analyzer energy discrimination. The software ran on a Digital VAX 3100 microcomputer and included automated crystallographic powder diffraction analysis. The most current powder diffraction database from the International Center for Diffraction Data Base (ICDD) was used. A copper $K\alpha$ radiation source was used for this study. Intensities were accumulated from 4 to 25 seconds per point at 0.03° 2 θ increments.

Assay for Prostaglandin E₂ (PGE₂) and Lactate Dehydrogenase (LD)

PGE₂ in macrophage supernatants was determined by a commercially available radioimmunoassay kit from PLA General Hospital, Beijing, China. Sensitivity for the assay was 25 pg/ml.

LD release by cultured macrophages was measured according to methods described by Morgenstern et al.^[4] The colorimetric estimation of the enzyme was determined from a standard curve.

Statistical Analyses

Data from two sets of parameters obtained from AM cultures were compared by a two-tailed t-test, and $p < 0.05$ was considered significant.

RESULTS/DISCUSSION

Elemental Composition of Coal Dust

Scanning electron microscopy in conjunction with energy-dispersive X-ray spectrometric analysis of each of the three coal dusts revealed that all dusts—meager, anthracite, and fat—consist of Al and Si (silicates) as common constituents. In addition, each dust exhibits elements that are characteristic of themselves. As seen in Figure 1, meager coal gives elemental peaks for aluminum, silicon; a small sulphur peak; and a very small titanium peak. Anthracite exhibits peaks for aluminum, silicon, and a large peak for sulfur, indicating that this is a high-sulfur-containing coal. A wavelength-dispersive X-ray spectrometry confirms these results. Anthracite contains the greatest amount of sulfur (1.5 wt%), followed by fat coal (0.53 wt%), and meager coal (0.47 wt%). Fat coal dust shows peaks for aluminum, silicon, sulfur, and a large peak for calcium.

Semiquantitative analysis by wavelength-dispersive X-ray spectrometry reveals that the three coal samples contain high carbon contents: fat coal, 96 wt%, anthracite 94 wt%, and meager coal, 93 wt%. There are small amounts of Mg, Na, K, Cl, and Fe. Iron contents are as follows: fat coal, 0.047 wt%; anthracite, 0.041 wt%; and meager coal, 0.040 wt%. Trace amounts of P, Mn, Ni, Cu, Zn, Ga, As, Se, Br, Sr, Zr, As, and Pb are present.

Mineralogic Composition of Coal Dust

Diffraction patterns were obtained for the three samples in the 2θ range from 5° to 90° . The d-spacing (Å)/relative intensity (counts per second) were automatically determined. The amorphous background is high for all three samples, reflecting their carbon contents.

In Figure 2, background-subtracted diffraction patterns are given for coal samples 1a, 2a, and 3a. X-ray diffraction assignments of kaolinite, muscovites, pyrite, calcite, and quartz are shown in Figure 2. All three coal samples consist of predominately aluminum silicates hydroxide with the chemical form $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$. The diffraction pattern for sample 1a, meager bituminous coal dust, is summarized as follows: (1) high kaolinite contents; (2) small amount of muscovite or potassium aluminum silicate hydroxide with chemical form $\text{KAl}_2(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH},\text{F})_2$; sample 2a, anthracite, mineral phases included kaolinite, pyrite FeS_2 . Muscovite is a small constituent of this sample. Kaolinite contents are also high in sample 3a fat coal dust. Additional enhanced lines indicate the existence of calcite CaCO_3 . Pyrite peaks in sample 3a are weak compared with sample 2a. All three coal dusts contain a small amount of quartz (crystalline SiO_2).

Cytotoxicity

Cytotoxicity is evident in AM cultures at 24 hours, with all three coal dust concentrations at 200 µg/ml. Controls without coal dust show 97.5% viability at 24 hours incubation (Figure 3). The AM cultured with three coal dusts exhibits significantly decreased viability compared with untreated controls ($p < 0.01$ or < 0.05). There is no significant difference in viability among three coal dusts ($p > 0.05$). Cytotoxicity is dose-dependent (200 µg/ml, 400 µg/ml, 800 µg/ml) for all three coal dusts (data not shown).

Release of Lactate Dehydrogenase and PGE₂

As seen in Figure 4, LD release increases in all three coal dusts compared with untreated controls ($p < 0.01$). Generally, this release is dose-related and increases over time (data not shown). Anthracite causes greater release of LD than fat coal ($p < 0.01$).

Figure 5 illustrates the influence of coal dust on the release of PGE₂ from AM. As is the case with LD, there is increased release of PGE₂ compared with untreated controls ($p < 0.01$ or < 0.05). The PGE₂ release is also dose-related (data not shown). Anthracite induces greater release of PGE₂ than fat coal ($p < 0.05$).

SUMMARY

This study demonstrates that all three coal dusts exert cytotoxicity on lung AMs and are capable of causing the release of LD and PGE₂. The release occurs with a small amount of minerals (4-7 wt% of coal samples) present in culture media. PGE₂ may exert potent modulating effects on AMs in the inflammatory response. Elevated levels of LD in rat bronchoalveolar lavage fluid have been reported to correlate well with the degree of known fibrogenic potential of different dusts in the lungs.^[5]

Several mechanisms have been proposed to support the role of macrophage products in the development and progression of silicosis and CWP. Such products include enzymes, cytokines,^[6] growth factors, and reactive oxygen species that may cause lung injury.^[3] This study shows that the three coal dusts contain pyrite (FeS₂). Huang et al.^[7,8] have shown that ferrous sulfate (FeSO₄) originated from oxidation of pyrite can reduce oxygen to produce reactive oxygen species in aqueous medium in vitro and can also inactivate alpha 1-antitrypsin, resulting in destruction of the lung parenchyma. These processes may play an important role in the development of emphysema in coal miners. Dalal et al.^[9] have proposed that higher concentrations of surface iron in coal dust may be involved in the generation of increased levels of hydroxyl radicals and lipid peroxidation. The hydroxyl radicals are highly reactive, causing peroxidation of cell membranes and cell injury, and may play an important role in the development of CWP. Finally, silica cannot be precluded as playing some role in the pathogenesis of CWP, since it is present at low concentrations in the three coal dusts.

In conclusion, this study demonstrates that the three coal dusts exhibit cytotoxicity on AMs and that the pathogenesis of CWP may be related to the grade of coal, including its elemental compositions and mineralogic components.

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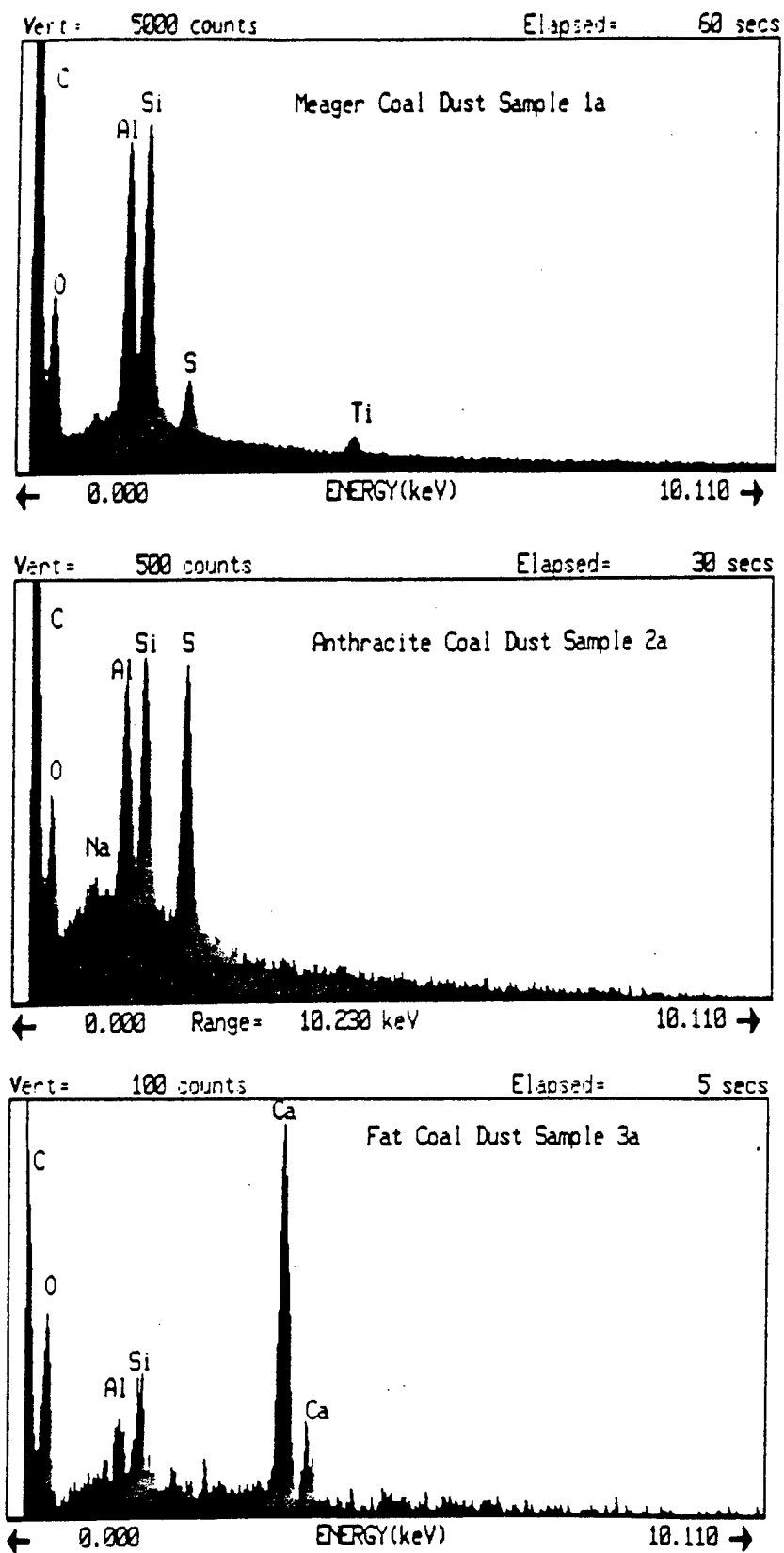
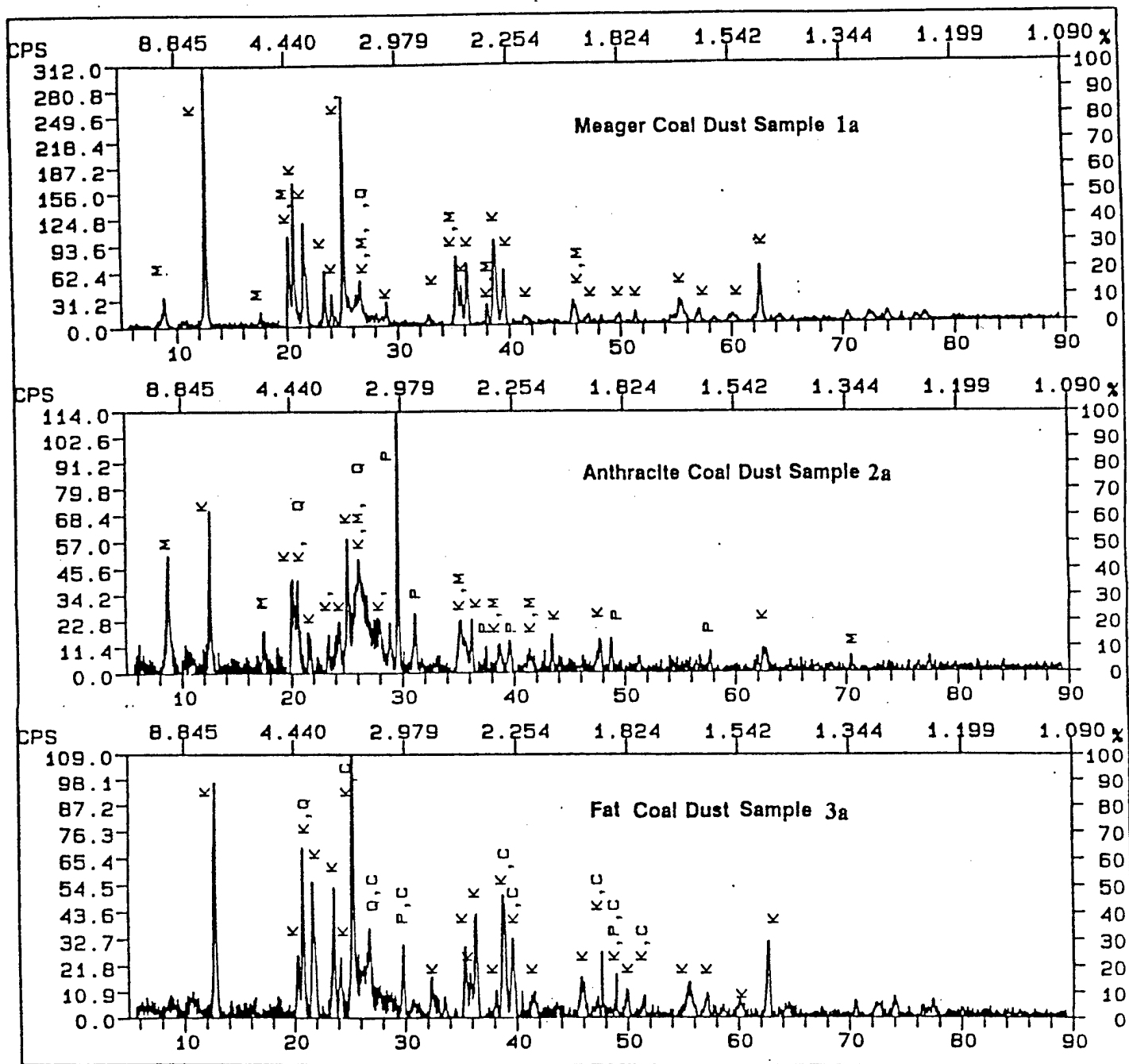
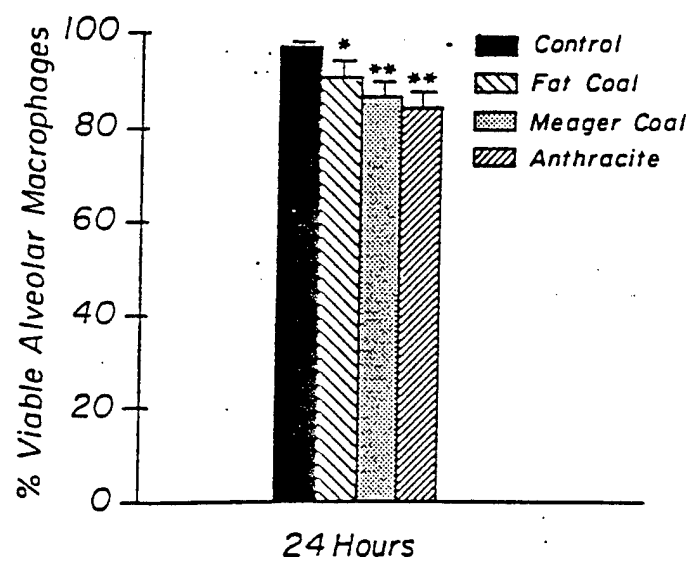


Figure 1. Energy-dispersive X-ray spectrometry of three coal dusts



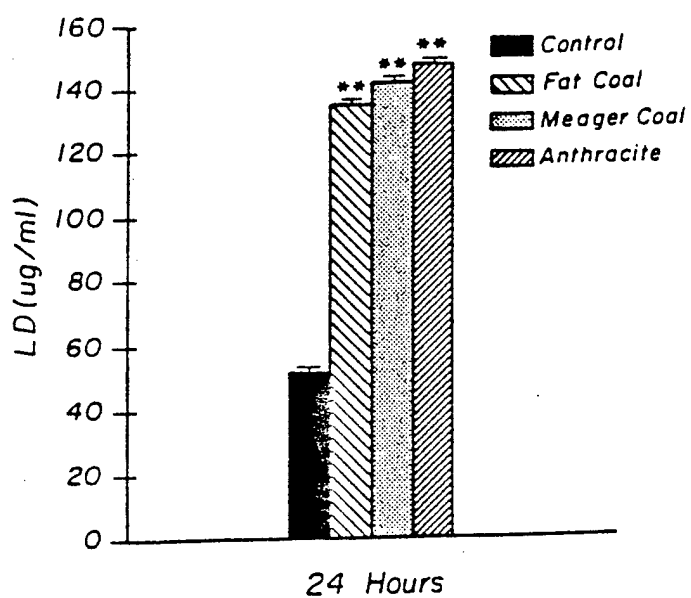
K-Kaolinite, M-Muscovite, P-Pyrite, C-Calcium, Q-Quartz

Figure 2. X-ray diffraction patterns for three coal dusts



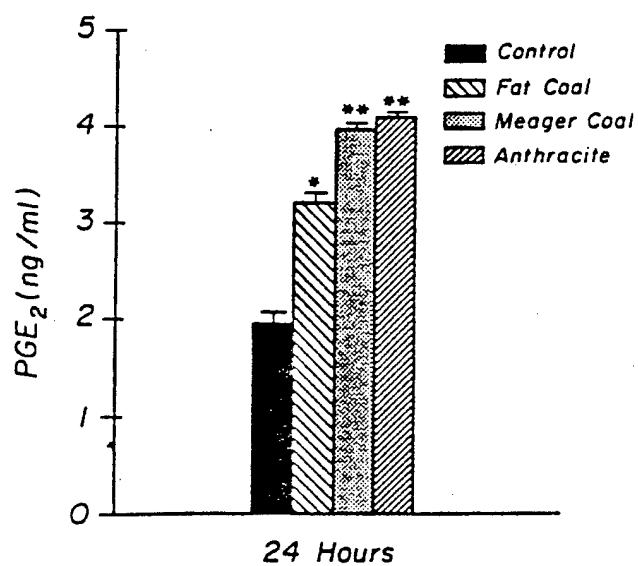
Legend: Each bar represents mean SE from 5 determinations
(* $p < 0.05$ vs. control ** $p < 0.01$ vs. control)

Figure 3. Cytotoxicity of three coal dusts on alveolar macrophages at coal dust concentration 200 $\mu\text{g/ml}$



Legend: Each bar represents mean SE from 5 determinations (** $p < 0.01$ vs. control)

Figure 4. Effects of coal dusts on LD release from alveolar macrophages at coal dust concentration 200 $\mu\text{g/ml}$



Legend: Each bar represents mean SE from 4 determinations.
(* $p < 0.05$ vs. control ** $p < 0.01$ vs. control)

Figure 5. Effects of coal dusts on PGE₂ release from alveolar macrophages at coal dust concentration 200 μ g/ml

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